

## CLAIMS

We claim:

1. An apparatus comprising:

- a) a substrate with a surface comprising a plurality of assay locations in a hybridization chamber, each assay location comprising a plurality of discrete sites;
  - b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;
- wherein said microspheres are distributed on each of said assay locations.

2. An apparatus according to claim 1 wherein each of said assay locations comprises a substantially similar set of bioactive agents.

3. An apparatus according to claim 1 wherein said substrate is a microtiter plate and each assay location is a microtiter well.

4. An apparatus according to claim 1 wherein each discrete site is a bead well.

5. An apparatus according to claim 1 wherein each of said subpopulations further comprise an optical signature capable of identifying said bioactive agent.

6. An apparatus according to claim 1 wherein each of said subpopulations further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

7. An apparatus comprising:

- a) a first substrate with a surface comprising a plurality of assay locations;
  - b) a second substrate comprising a plurality of array locations, each array location comprising discrete sites;
  - c) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;
- wherein said microspheres are distributed on each of said array locations; and
- d) a hybridization chamber configures so as to receive said second substrate.

8. An apparatus according to claim 7 wherein said first substrate is a microtiter plate.

9. An apparatus according to claim 7 or 8 wherein said second substrate comprises a plurality of fiber optic bundles comprising a plurality of individual fibers, each bundle comprising an array location, and each individual fiber comprising a bead well.

10. An apparatus according to claim 9, wherein said hybridization chamber further comprises at least one component port.

11. An apparatus according to claim 7 wherein each of said subpopulations further comprise an optical signature capable of identifying said bioactive agent.

12. An apparatus according to claim 7 wherein each of said subpopulations further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

13. A hybridization chamber comprising:

- a) a base plate wherein a base cavity for holding a first array component is formed in said base plate;
- b) a lid comprising at least one component port for immobilizing a second array component;
- c) a sealant between said base plate and said lid.

14. The chamber according to claim 13, wherein said second array component is a fiber optic bundle.

15. The chamber according to claim 13 further comprising at least one alignment feature.

16. The chamber according to claim 15, wherein said at least one alignment feature is a male and female fitting.

17. The chamber according to claim 13, further wherein said first array component is a microtiter plate.

18. The chamber according to claim 13 further comprising at least one fluid handling device.

19. A method of decoding an array composition comprising

- a) providing an array composition in a hybridization chamber, said array composition comprising:

- i) a substrate with a surface comprising a plurality of assay locations, each assay location comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; wherein said microspheres are distributed on said sites;  
b) adding a plurality of decoding binding ligands to said array composition to identify the location of at least a plurality of the bioactive agents.

20. A method of decoding an array composition comprising

a) providing an array composition in a hybridization chamber, said array composition comprising:

i) a substrate with a surface comprising a plurality of array locations, each array location comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said sites;

b) adding a plurality of decoding binding ligands to said array composition to identify the location of at least a plurality of the bioactive agents.

21. A method according to claim 19 or 20 wherein at least one subpopulation of microspheres comprises an identifier binding ligand to which a decoding binding ligand can bind.

22. A method according to claim 19 or 20 wherein said decoding binding ligands bind to said bioactive agents.

23. A method according to claim 19 or 20 wherein said decoding binding ligands are labeled.

24. A method according to claim 19 or 20 wherein the location of each subpopulation is determined.

25. A method of determining the presence of one or more target analytes in one or more samples comprising:

a) contacting said sample with a composition comprising:

i) a substrate with a surface comprising a plurality of assay locations, each assay location comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent, wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres;

b) incubating in a hybridization chamber; and

c) determining the presence or absence of said target analyte.

26. A method of determining the presence of one or more target analytes in one or more samples comprising:

- a) adding said sample to a first substrate comprising a plurality of assay locations, such that said sample is contained at a plurality of said assay locations;
- 5 b) contacting said sample with a second substrate comprising:
  - i) a surface comprising a plurality of array locations, each array location comprising discrete sites; and
  - ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent, wherein said microspheres are distributed on said
  - 10 surface such that said discrete sites contain microspheres;
- b) incubating in a hybridization chamber; and
- c) determining the presence or absence of said target analyte.

27. A method of mixing solutions in an array format comprising:

- 15 a) providing a hybridization chamber comprising:
  - i) a base plate comprising holes, wherein at least two of said holes are joined by a channel;
  - ii) a membrane;
  - ii) a lid comprising at least one component port for immobilizing an array component;
  - 20 iii) a sealant between said base plate and said lid;
- b) applying a vacuum to said membrane whereby wells are formed in said membrane;
- c) providing a solution to said membrane whereby said solution enters at least one well;
- d) intermittently applying vacuum to said membrane, whereby said solution is mixed.

28. The method according to claim 15, wherein said solution enters a plurality of said wells.

29. A method of detecting the presence or absence of a plurality of target analytes, comprising
- (a) providing a first substrate with a surface comprising a plurality of assay wells, wherein said assay wells contain sample solutions each having a plurality of target analytes;
  - (b) providing a second substrate comprising a plurality of array locations, each array location
  - 5 comprising a plurality of discrete sites, wherein said sites comprise different bioactive agents;
  - (c) dipping said array locations into said assay wells under conditions suitable for binding of said target analytes to said bioactive agents, thereby processing said sample solutions in parallel; and
  - (d) detecting the presence or absence of said target analytes.
- 10 30. The method of claim 29, wherein said target analytes comprise nucleic acids or nucleic acid analogs.
31. The method of claim 30, wherein said nucleic acids comprise single nucleotide polymorphisms.
- 15 32. The method of claim 31, comprising multiplex PCR amplification of said single nucleotide polymorphisms and subsequent binding to said bioactive agents.
33. The method of claim 30, wherein said nucleic acids are labeled with fluorochromes during PCR amplification.
- 20 34. The method of claim 29, wherein said bioactive agents are selected from the group consisting of peptides, peptide structural analogs, saccharides, fatty acids, steroids, purines, and pyrimidines.
35. The method of claim 29, wherein said array locations comprise from 10,000,000 to
- 25 2,000,000,000 bioactive agents per square centimeter.
36. The method of claim 29, wherein said array locations comprise from 100,000 to about 10,000,000 bioactive agents per square centimeter.
- 30 37. The method of claim 29, wherein said array locations comprise from 10,000 to about 100,000 bioactive agents per square centimeter.
38. The method of claim 29, wherein said bioactive agents are directly coupled to said array
- 35 locations.
39. The method of claim 29, wherein said bioactive agents are attached to microspheres and wherein said microspheres are associated with said array locations.
40. The method of claim 29, wherein said target analytes comprise decoder binding ligands.
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41. The method of claim 29, wherein said target analyte is labeled.
42. The method of claim 41, wherein said label comprises an optical label.
- 5 43. The method of claim 42, wherein said optical label comprises a fluorochrome.
44. The method of claim 29, wherein said detecting is done through the use of a change in optical signature.
- 10 45. The method of claim 29, further comprising quantitating differences in concentrations of said target analytes
46. The method of claim 45, further comprising quantitating a specific mRNA.
- 15 47. The method of claim 46, comprising quantitating said specific mRNA in the presence of total cellular mRNA.
48. The method of claim 29, wherein said assay wells comprise wells of a microtiter plate.
- 20 49. The method of claim 29, comprising 96 wells.
50. The method of claim 29, comprising 384 wells.
51. The method of claim 29, comprising 1536 wells.
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